

A QUANTITATIVE STUDY OF
REACTIONS TO ELECTRICITY IN
AMOEBA PROTEUS

WILLIAM F. HAHNERT

DISSERTATION SUBMITTED TO THE BOARD OF
UNIVERSITY STUDIES OF THE JOHNS HOPKINS
UNIVERSITY IN CONFORMITY WITH THE RE-
QUIREMENTS FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY

1931

Reprinted from
PHYSIOLOGICAL ZOÖLOGY
Vol. V, No. 4, October, 1932



Physiological Zoölogy

Vol. V

OCTOBER 1932

No. 4

A QUANTITATIVE STUDY OF REACTIONS TO ELECTRICITY IN AMOEBA PROTEUS¹

(Eleven figures)

WILLIAM F. HAHNERT

Zoölogical Laboratory, The Johns Hopkins University

INTRODUCTION

IT IS well known that electrical changes profoundly affect various vital processes. Among the most striking of these is the effect on protoplasmic streaming. Becquerel (1837) concluded from the results of observations on *Chara* (1) that a constant current causes retardation and then cessation of streaming, (2) that the time required to produce these effects depends upon the strength of the current, (3) that after streaming is stopped by a weak current, it soon begins again and usually attains its original velocity but never a greater velocity than existed before excitation, and (4) that after streaming is resumed, a stronger current is required to cause it to stop again. Results confirming the first two of these conclusions were obtained by Jürgensen (1861) in *Vallisneria*, Hörmann (1898) in *Nitella*, and Koketsu (1923) in *Chara*. Koketsu maintains that a constant current causes increase in the viscosity of the protoplasm, a view previously proposed by Chiffot and Gautier (1905) and Bersa and Weber (1922) to account for the effect produced by the current in certain other plant cells. Velten (1876) and Ewart (1903) hold that weak currents frequently accelerate streaming; and Hörmann

¹ This investigation was suggested by Professor S. O. Mast, to whom the author is indebted also for invaluable assistance and criticism, especially in the preparation of the manuscript.

(1898) contends that after cessation has been produced once, a weaker current suffices to produce it again.

The investigation of the effect of a galvanic current on protoplasmic streaming and on locomotion in the lower animals has yielded similar results. Kühne (1864) and Engelmann (1869) observed that in *Amoeba* a constant current causes cessation of streaming and that the time required to produce it depends upon the strength of the current. Kühne asserts in support of Becquerel that after a few induction shocks a stronger current is required to produce a reaction, but he holds in opposition to Becquerel that single weak shocks frequently accelerate the rate of streaming.

Verworn (1896) demonstrated that *Amoeba* orients definitely in a galvanic current and migrates toward the cathode. This has been abundantly confirmed; it is ascribed by Verworn (1896) to anodal contraction, by Carlgren (1900) to localized changes in water content within the organism due to endosmotic streaming, by Coehn and Barratt (1905) to cataphoresis, by Hirschfeld (1909) to reduction in surface tension on the cathodal side, by McClendon (1911) to increase in surface tension on the anodal side, and by Luce (1926) and Mast (1931) to local decrease in the elastic strength of the plasmagel on the cathodal side of the organism.

Luce (1926) made a detailed study concerning the processes involved in orientation to electricity in *Amoeba*. He obtained some very interesting results concerning the relation between the strength of the current and the directness of orientation. He says: "Orientation is slow and uncertain with weak currents and becomes more rapid as the current increases until direct reversal of flow takes place." He concludes: "Orientation is brought about by the formation of pseudopods on the cathodal side, the effect of the current being to cause tendencies toward solution on that side." Mast (1931) comes to similar conclusions on the basis of results obtained in observations on the effect of alternating current.

The evidence at hand shows clearly that there is great diversity of opinion concerning the action of electricity on the lower organisms. It shows that *Amoeba* orients and migrates toward the cathode and that the process of orientation is directly dependent upon the strength of the current, but it does not show the relation between the

strength of the current and the rate of migration or the reaction-time in the response to electricity. The present investigation treats of these problems and is primarily concerned with the quantitative relation between the stimulating agent and the subsequent response, as indicated by changes in the rate and direction of movement.

MATERIAL AND METHODS

All of the observations described in the following pages were made on specimens derived from a pure clone culture of *Amoeba proteus* Leidy. These amoebae were cultivated and studied in synthetic solutions of known salt content and hydrogen-ion concentration. Chemi-

TABLE I

SALT SOLUTION USED FOR CULTURING *Amoeba proteus*

NaCl.....	0.08	gram
NaHCO ₃	0.004	gram
KCl.....	0.004	gram
CaCl ₂	0.004	gram
CaH ₄ (PO ₄) ₂	0.002	gram
Sorensen's phosphate mixture:		
Na ₂ HPO ₄ , M/15.....	2 cc.	} = pH 6.64
KH ₂ PO ₄ , M/15.....	3 cc.	
Redistilled water to.....	1,000	cc.

cals of the highest purity and water redistilled in pyrex glass were used in all solutions.

The culture method may be described as follows: A salt solution was prepared as indicated in Table I. About 100 cc. of this solution was poured into each of a number of finger bowls, after which one or two grains of polished rice and specimens of *Amoeba proteus* and *Chilomonas paramecium* were added to each. The rice soon became covered with a mold, on and among the strands of which numerous specimens of *Amoeba* and *Chilomonas* aggregated. New cultures were set up by transferring one of these grains with its mold and organisms to a finger bowl containing fresh solution.

In order to ascertain the effect of the constant electric current on locomotion and other processes in *Amoeba*, it is necessary to vary this factor in a known way without varying any other factors. This cannot be accurately done in the culture fluid, because in it the elec-

trolytic decomposition by the current is great and the general excitability of the organism is reduced. For this reason, all of the observations were made in another synthetic solution, designated as "test solution." Its composition is given in Table II. It differs from the culture solution as follows: (1) Sodium bicarbonate, sodium chloride, potassium chloride, and calcium acid phosphate are eliminated. (2) The phosphate buffer and the calcium chloride are more concentrated. (3) The ratio of Na, K, and Ca is 12:15:2, as compared with 38:9:1 in the culture solution. (4) The total salt content is only about one-half that of the culture fluid.

The experiments were carried out in a darkened room, observations being made with a compound microscope. The electricity used

TABLE II

SALT SOLUTION USED IN THE EXPERIMENTAL TESTS

CaCl ₂	0.008 gram	
Sorensen's phosphate mixture:		
Na ₂ HPO ₄ , M/15.....	4 cc.	} = pH 6.64
KH ₂ PO ₄ , M/15.....	6 cc.	
Redistilled water to.....	1,000 cc.	

was drawn from a 110-volt direct-current lighting circuit, passed through two MacLagan rheostats to a reversing switch, then through a mercury contact key to sheet-platinum electrodes. A very accurate Rawson milliammeter was placed in line in front of the reversing switch (Fig. 1, A). The observation slide used in these tests contained a rectangular glass trough, 70 mm. long, 10 mm. wide, and 5 mm. deep; it was constructed by cementing strips of glass slide on the surface of a glass slide. Two porcelain plates, 0.4 mm. in thickness, were cemented across the trough, dividing it into three compartments—two for the electrodes and one for the amoebae (Fig. 1, B and C). Hereafter the center compartment of this trough will be referred to as the "observation compartment" and the end ones as the "electrode compartments."

In making the experiments, the three compartments on the observation slide were filled with the test solution; then about twenty amoebae were taken from a stock culture and put into a pyrex glass

beaker containing redistilled water and left 15 minutes.² By the end of this time, nearly all of the amoebae were radiate in form; these were removed and transferred through four changes of test solution, allowing them to remain 15 minutes in the last for adjustment. By

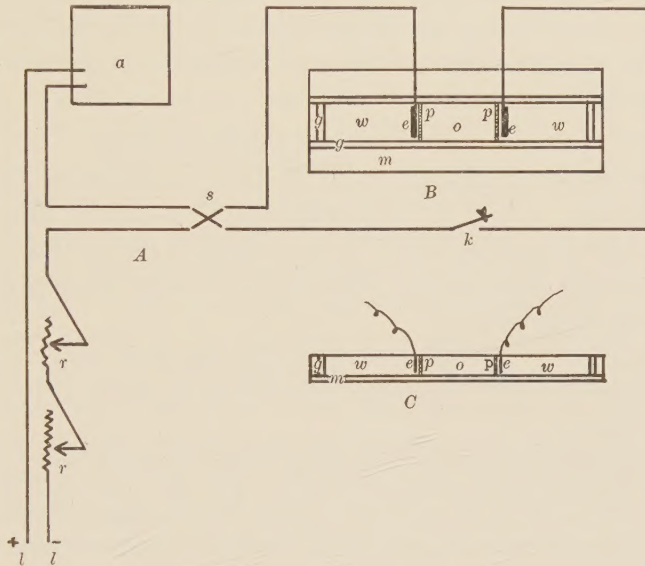


FIG. 1.—A, Diagram showing arrangement of apparatus used in the study of reactions to a galvanic current in *Amoeba*. B, The observation slide used in this study, viewed from above. C, The same viewed from the side. *l-l*, Current source; *r-r*, rheostats in series; *a*, ammeter; *s*, reversing switch; *k*, mercury contact key; *e-e*, electrodes; *w-w*, electrode compartments; *o*, observation compartment; *p-p*, porcelain plates; *m*, microscope slide; *g-g*, glass strips forming walls of trough.

this time many of them were fairly monopodal; two to five of these were then transferred to the middle of the center compartment on the observation slide. A cover glass was now placed over this compartment and the observation slide put upon the stage of the compound microscope, after which the electrodes were inserted into the solution in the end compartments. As soon as the amoebae became attached to the bottom, a specimen which was monopodal and moving

² The wash in redistilled water was used only in the experiments on the rate of locomotion. There is some indication that it may play a rôle in causing the amoebae to assume the monopodal form more quickly and to retain it for a longer period of time.

in the desired direction was selected, then the polarity of the electrodes was adjusted as desired by means of the reversing switch. The current³ was now turned on by closing the contact key, and the reaction of the specimen observed.

Under favorable conditions the response in *Amoeba* to a constant current is sharp and precise, so that it can be clearly seen and conveniently measured. Most of the results obtained are presented in the form of figures, tables, and graphs.

LOCOMOTION IN UNSTIMULATED AMOEBAE

Before discussing the effect of electricity on movement in *Amoeba*, a brief account of locomotion in unstimulated specimens will prove helpful. For the present study, such an account should treat of the structure of the organism, the processes involved in locomotion, and the rate of locomotion.

The structure of *Amoeba proteus* and the processes involved in its locomotion have been thoroughly discussed by Mast (1926). He says (p. 411):

An active monopodal amoeba of the proteus type may then be looked upon as consisting essentially of an elongated relatively rigid tube (plasmagel) with the one end, the anterior, covered with a thin sheet (plasmagel sheet) of porous substance having a low elastic strength, and the other, the posterior, with a thicker sheet having a higher elastic strength, a hypertonic fluid substance (plasmasol) filling the cavity of the tube, a very thin membrane (plasmalemma) of very low elastic strength covering the tube, and a thin layer of liquid between the surface membrane and the tube nearly everywhere except in the regions adjoining those where the membrane is in contact with the substratum (this layer is thick at the tip of active pseudopods and is called hyaline cap). And locomotion in such an amoeba may be looked upon as being essentially due to contraction of the thick sheet covering the posterior end, forcing the column of fluid, the plasmasol, in the tube forward against the thin sheet at the anterior end, stretching it and pushing it forward together with the surface membrane, which, owing to attachment to the substratum and to the adjoining wall of the tube, slides forward over the wall of the tube above, resulting in forward movement of the whole system.

Concerning the rate of locomotion in *Amoeba proteus*, the data are very meager. Schaeffer (1920) says that it moves about $600\ \mu$ per

³ The strength of the current was controlled by means of the rheostats; it is expressed in delta (δ), a unit which indicates a current of $1/1,000$ milliamperes per square millimeter of cross-sectional area of the solution in the observation compartment.

minute. Schwitalla (1924) made a detailed study on the effect of temperature on the rate of locomotion, and states that the average rate of all the amoebae studied at various temperatures was 625μ per minute. This must, however, be a misprint, since none of the data presented in his paper shows a rate greater than 250μ per minute. Hopkins (1928), in observations on the effect of hydrogen-ion concentration, obtained a maximum rate of about 150μ per minute. Pantin (1924) maintains that the maximum rate in "limax," a marine amoeba, is about 180μ per minute and that, if the conditions of the medium are kept constant, the rate in any individual is constant to within 1-5 per cent for at least 24 hours. Schwitalla contends that this does not obtain for *Amoeba proteus*. He says that in this form the rate usually varies greatly, only rarely remaining the same in two successive minutes and frequently changing from zero to the maximum, or the converse, in a very few minutes. By referring to Figure 3 in Schwitalla's paper, it will be seen that there was great variation in the form of the amoebae used; i.e., some were nearly monopodal, others bipodal, and still others multipodal. It may be, therefore, that the rate observed by him was specifically correlated with the form. If this is true, variation in rate can be greatly reduced by selecting amoebae of the same form. If this can be done, it will obviously greatly facilitate observations on the effect of environmental factors on the rate. Further observations on the rate of locomotion in unstimulated specimens were consequently made as follows:

Two to ten amoebae, prepared as described above, were put into test solution in the observation compartment. As soon as they became attached to the bottom, a monopodal specimen was selected; its rate of locomotion was then ascertained by projecting the image of the specimen upon a sheet of paper by means of a camera lucida, marking the position of the posterior end of the organism at intervals of 1 minute over a long period of time, and measuring the distance between successive positions with a metric rule. The image as projected upon the paper was magnified 85 times, making the rate recorded 85 times the actual rate.

The rate of locomotion was thus ascertained for numerous specimens, but the records of only ten are used as the basis for this dis-

cussion. These ten amoebae were observed at minute intervals for a total of 177 minutes, so that, on an average, each amoeba was under observation 18 minutes.

The results obtained are presented in Figure 2 and Table III. Figure 2 is the actual camera lucida record of locomotion obtained in

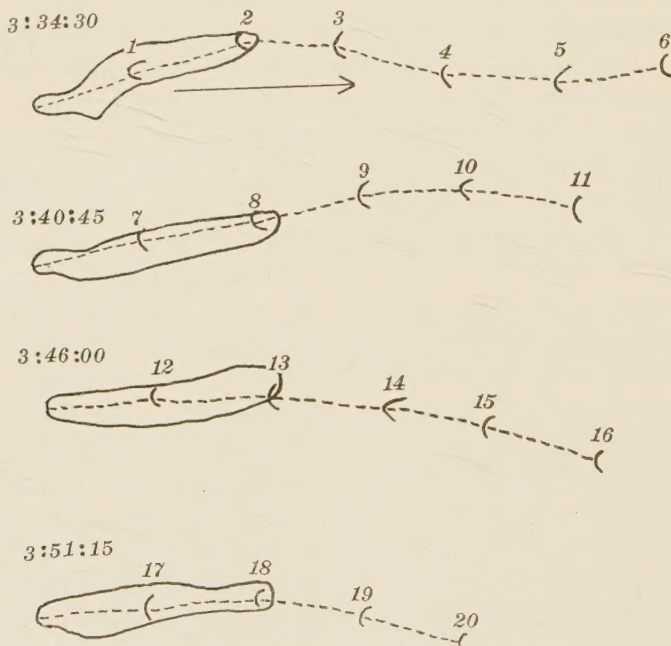


FIG. 2.—Camera lucida drawings showing record of locomotion in an unstimulated amoeba. Arrows, direction of locomotion; dotted line, path taken; curved cross-lines, positions of the posterior end at intervals of 1 minute.

one specimen. The figure shows that the record of the positions of the organism is clear, the method of measurement accurate, and the rate of locomotion uniform. In fact, in 20 consecutive minutes the mean apparent rate of locomotion in this specimen was 21.47 mm. per minute, with a standard deviation of only 1.156 mm. per minute, or 5.38 per cent.

Table III contains the average of the results obtained in ten specimens. By referring to the table, it will be seen at once that the rate of locomotion over the whole period of time was remarkably con-

stant; indeed, the mean apparent rate was 20.7 mm. per minute, with a standard deviation of only 0.74 mm. per minute, or 3.57 per cent. It may be of interest to add that the average actual rate of locomotion in these ten unstimulated monopodal amoebae was about 250 μ per minute—less than one-half that given by Schaeffer.

The results presented indicate clearly that the process of ascertaining the rate of locomotion in *Amoeba proteus* is greatly simplified by using monopodal specimens and that the rate in such specimens in constant conditions is remarkably constant.

TABLE III

AVERAGE APPARENT RATE OF LOCOMOTION IN TEN UNSTIMULATED
MONOPODAL AMOEBAE AT 19°–22° C.

To obtain actual rate in millimeters per minute, divide apparent rate by 85.

Mean apparent rate, 20.7 mm. per minute.

Standard deviation, 0.74 mm. per minute, or 3.57 per cent.

Periods (1 Minute Each)	Rate (Millimeters per Minute)	Periods (1 Minute Each)	Rate (Millimeters per Minute)
1.....	21.2	13.....	20.9
2.....	21.3	14.....	21.3
3.....	20.4	15.....	19.8
4.....	21.4	16.....	21.1
5.....	21.1	17.....	19.8
6.....	20.8	18.....	20.2
7.....	22.2	19.....	19.5
8.....	21.3	20.....	20.0
9.....	21.5	21.....	20.6
10.....	20.8	22.....	20.0
11.....	20.6	23.....	19.5
12.....	20.2		

EFFECT OF CONSTANT CURRENT DENSITY ON THE RATE OF LOCOMOTION

Having now demonstrated that under certain conditions the rate of locomotion in *Amoeba proteus* is fairly uniform over a considerable period of time, we are in a better position to consider the effect of a galvanic current on it. Let us consider first the effect produced by currents of constant density and then that produced by sudden changes in current density. Observations on the rate of locomotion in currents of constant density were made as follows:

Two to ten amoebae prepared as described above were transferred to the middle of the observation compartment and observed until

one was found which was moving in monopodal form toward the cathode. The rate of movement at the posterior end of this specimen was now recorded, by the camera lucida method, each minute for 3 or 4 minutes; the circuit was then closed, after which the rate was recorded each minute as long as the specimen continued to move in

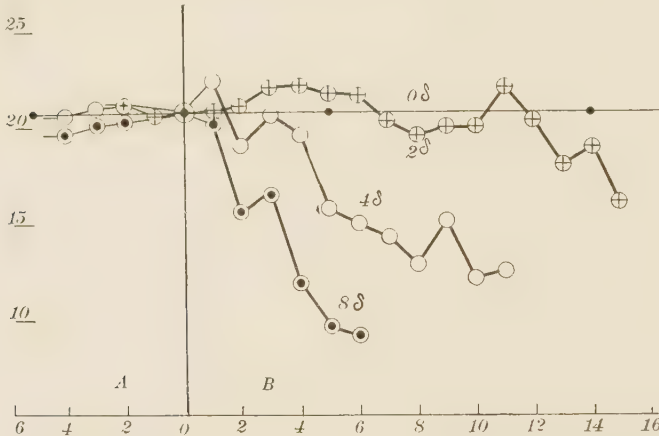


FIG. 3.—Graphs showing comparative effect of different densities of current on the rate of locomotion. Abscissae, time in minutes, (A) before and (B) during the passage of current; ordinates, apparent rate of locomotion. To obtain actual rate in millimeters per minute, divide apparent rate by 85.

monopodal form toward the cathode.⁴ The distances traveled per minute before and during the passage of the current were then measured and compared. In this manner the effect of the current on the rate of locomotion was ascertained in numerous specimens.

The results obtained are presented in Figure 3. In this figure each curve represents the average apparent rate obtained in ten specimens in the stated current density. The curves are superimposed, so that at the time when the circuit was closed they coincide on the

⁴ In the experiments requiring prolonged passage of current, the porcelain plates did not entirely prevent the products of electrolytic decomposition about the electrodes from diffusing into the observation compartment. These products increase the hydrogen-ion concentration of the solution surrounding the amoebae and thereby greatly affect their activities. Diffusion of these products into the observation compartment was greatly delayed by placing a fresh agar strip, 2-5 mm. thick, in each electrode compartment between the electrode and the porcelain plate before each test.

straight line; this line represents the average apparent rate calculated from all the readings obtained in ten unstimulated individuals.

The figure shows that the rate of locomotion in all cases before the circuit was closed was fairly uniform; that in a weak current (2δ), it increased above the average and remained so for 6 minutes, then gradually declined; that in a medium current (4δ), it increased above the average during the first minute, then declined gradually, but more rapidly than in the weak current; and that in a strong current (8δ), it declined slightly during the first minute, then rapidly in succeeding minutes. This clearly indicates that both the strength and the duration of the current are effective in producing changes in the rate of locomotion.

The most conspicuous feature of these curves is the ultimate trend downward; less conspicuous, but equally significant, is the initial trend upward. The question now arises as to how the current acts on the organism to produce these opposite effects on the rate of locomotion.

Numerous investigators have observed that, during the passage of a weak constant current, *Amoeba* first contracts and becomes globular, then expands and resumes its original form, and sometime later undergoes another contraction, which continues until the organism disintegrates. These changes in form greatly influence the rate of movement of different parts of the amoeba. In the contraction of a monopodal amoeba, it is evident that the rate of movement of the posterior end will increase rapidly at first, then gradually fall to zero as the globular form is approximated, and that in subsequent expansion the rate of the posterior end will gradually increase as the monopodal form is approximated, at which time the original rate will be restored. It is evident, also, with such a system that a uniform rate of movement over a period of time is possible only when the organism is in a monopodal form, since locomotion does not occur in globular amoebae.

On the basis of these facts, a sharp rise in a curve, followed by a sharp decline, indicates an injurious contraction of the organism; a gradual rise, followed by a plateau, indicates an increase in the rate of locomotion of the organism as a whole; and a gradual decline, followed by a plateau, indicates either a decrease in rate or an elonga-

tion of the individual. All of these principles are illustrated in the 2 δ curve, where the initial rise and plateau show an increased rate, the gradual fall and plateau a decreased rate, and the second rise and fall contraction leading to disintegration. Further evidence in support of this interpretation will be presented in the following section.

As stated in the introduction, Becquerel (1837) holds that in *Chara* the passage of a galvanic current never causes increase in the rate of protoplasmic streaming. Contrary results were obtained by Velten (1876) and Ewart (1903) in plants and by Kühne (1864) in sluggish amoebae. The results presented above are also to the contrary and offer quantitative evidence that in *Amoeba* a weak constant current does increase the rate of protoplasmic streaming, that is, locomotion. Discussion concerning the processes involved in the increase in the velocity of movement in *Amoeba* will be deferred until certain other data have been presented.

The results as a whole show clearly that a weak constant current causes an initial increase in the rate of locomotion in *Amoeba proteus*, that this increase is later followed by a decline, and that the stronger the current the shorter the time required to cause the increase and the subsequent decrease in the rate of locomotion.

EFFECT OF SUDDEN CHANGE IN CURRENT DENSITY ON THE RATE OF LOCOMOTION

It was repeatedly observed in the tests just reported that the rate of movement of the posterior end in *Amoeba* suddenly increases immediately after the circuit is closed and that this effect becomes more evident as the current density increases. A detailed study was made therefore to ascertain more precisely at what time after making the current this increase in rate occurs and the relation between the magnitude of the increase and the strength of the current.

The methods used in ascertaining these relations are essentially like those used in the experiments described above. The amoebae and the observation slide were similarly prepared, after which the rate of locomotion was ascertained by means of drawings made with a camera lucida. In these tests, however, the positions of both anterior and posterior ends of the organisms were recorded, and the records were made at quarter-minute intervals. Not only were the posi-

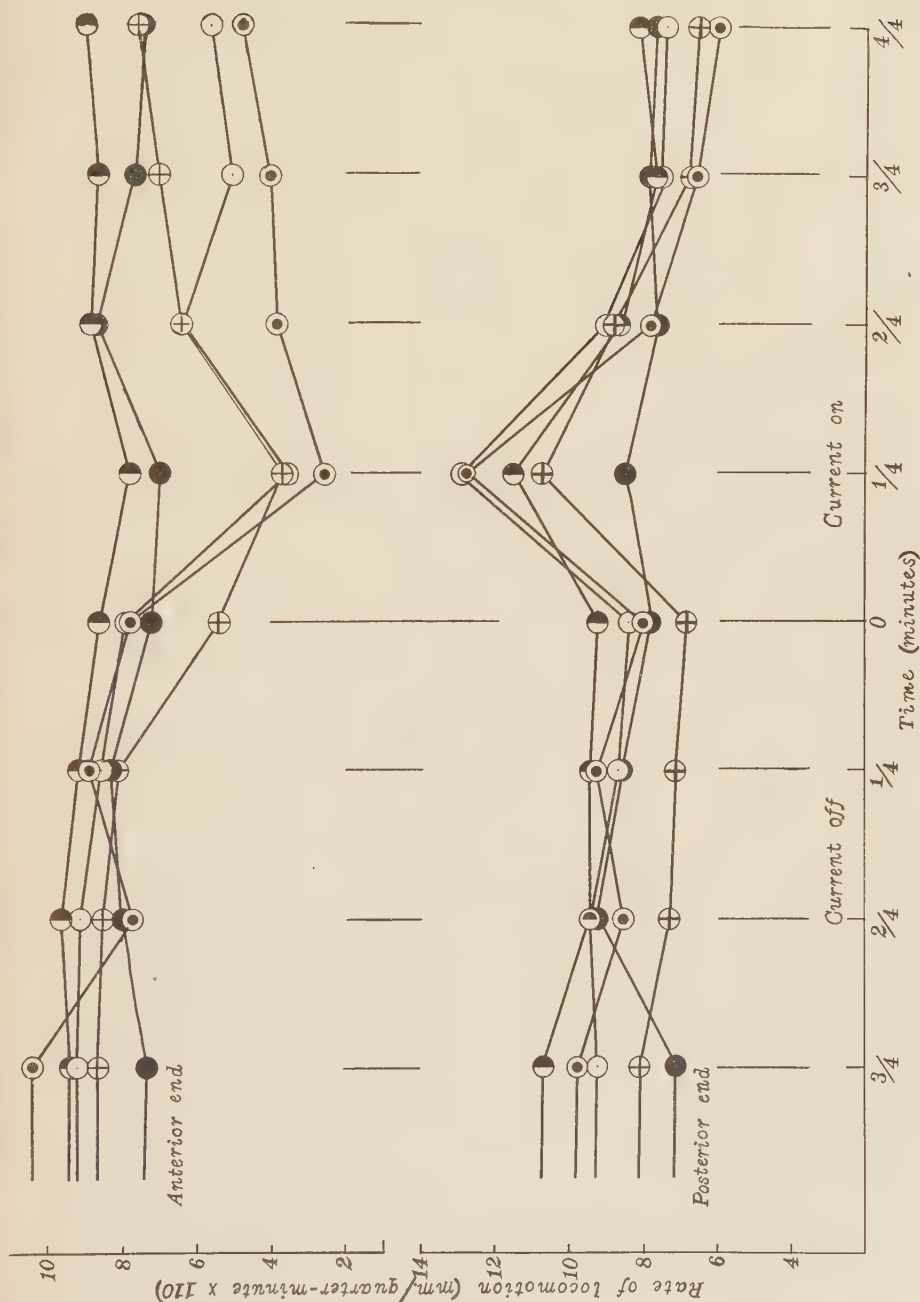


FIG. 4.—Graphs showing the immediate effect of different densities of current on the rate of movement at the two ends of amoebae. Upper group, anterior end; lower group, posterior end; ●, 4δ; ⊕, 8δ; ○, 12δ; ⊙, 16δ; abscissae, time in minutes; ordinates, apparent rate of movement in millimeters per quarter-minute. To obtain actual rate in millimeters per minute, divide apparent rate by 110 and multiply by 4.

tions recorded more frequently, but they were also recorded more accurately; the latter was accomplished by means of a more powerful lens system, which gave a magnification of 110 diameters. Because of the narrowness of the field of vision with this magnification and because of the disconcerting action of strong currents on the monopodal form of amoebae, each individual was observed for only 2 minutes—1 minute before making the current and 1 minute during the passage of the current. In this manner the effect of sudden

TABLE IV

EFFECT OF CURRENTS OF DIFFERENT DENSITIES ON THE RATE OF LOCOMOTION AT THE ENDS OF *Amoeba* IMMEDIATELY AFTER THE CIRCUIT IS CLOSED

A, anterior end; P, posterior end; —, decrease in rate; +, increase in rate; numbers, average apparent distance traveled in millimeters per quarter-minute.

To obtain actual rate in millimeters per minute, divide apparent rate by 110 and multiply by 4.

RATE DURING LAST INTERVAL BEFORE CLOSING CIRCUIT		CURRENT DENSITY MADE	RATE DURING FIRST INTERVAL AFTER CLOSING CIRCUIT		CHANGE IN RATE DURING THE INTERVAL	
A	P	δ	A	P	A	P
7.25	7.85	1	7.00	8.50	-0.25	+0.65
8.65	9.25	4	7.80	11.30	-0.85	+2.05
5.44	6.85	8	3.72	10.75	-1.72	+3.90
7.95	8.40	12	3.60	12.85	-4.35	+4.45
7.85	8.00	16	2.60	12.80	-5.25	+4.80

changes in current density on the rate of movement was ascertained for numerous specimens.

The results obtained are presented in Figures 4, 5, and 6 and Table IV. In Figure 4 the rate of movement at the anterior and the posterior end of the same organisms is given in separate series of graphs, each graph in each series representing the average apparent rate obtained in ten specimens in the stated current density.

By referring to the figure, it will be seen that the rate of locomotion in all cases before the circuit was closed was fairly constant; that immediately after the current was made, it changed suddenly (i.e., within 15 seconds), as indicated by decrease in rate at the anterior end and increase in rate at the posterior end; that the changes in rate were greater the stronger the current used; and that in succeeding

periods of time the rate at either end of the organisms tended to return to that which existed before stimulation.

Some of these facts are more evident in the results presented in Figure 5, especially the uniform rate before stimulation, the sudden

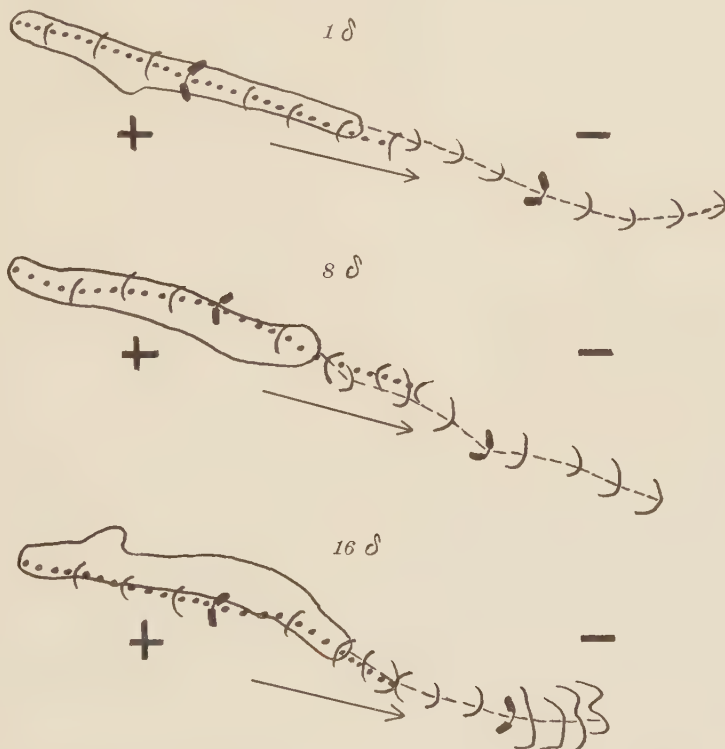


FIG. 5.—Camera lucida drawings showing the rate of locomotion in amoebae, for 1 minute before and 1 minute during the passage of current of different densities. Arrows, direction of movement; dotted line, path taken; curved cross-lines, position of the ends at successive quarter-minute intervals; heavy curved lines, position of the ends when the current was made; +, anode; —, cathode. Note that increase in rate at the anodal end and decrease in rate and expansion at the cathodal end occurred during the first quarter-minute after the current was made.

change in rate at the time of stimulation, and the tendency to resume the original rate during the passage of the current. By referring to this figure, it will be noted that the duration of the changes in rate is limited largely to the first quarter-minute after the circuit was closed and that during this period the form of the anterior end

changes considerably, i.e., it becomes broader. The significance of this will be shown presently.

The relation between the magnitude of the change in rate and the current density is still more clearly shown in Table IV, in which the

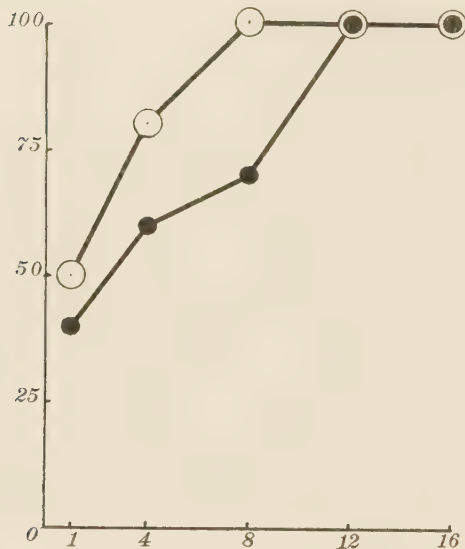


FIG. 6.—Graphs showing the relation between current density and the percentage of amoebae in which the rate of movement increased at the anodal end and decreased at the cathodal end during the first quarter-minute of passage of current. Abscissae, current density (δ); ordinates, percentage of specimens that respond; ○, anodal response; ●, cathodal response.

distance traveled by the amoebae in the last quarter-minute before passage of current is compared with that traveled in the first quarter-minute during the passage of current. The table shows that in every density tested the rate at the anterior end decreased, while that at the posterior end increased, immediately after the current was made; that the changes in rate were greater, the stronger the current made; and that in all densities except the strongest, the change in rate was greater at the posterior than at the anterior end. These results confirm the earlier observation that on stimulation

by a constant current, *Amoeba* contracts. They show, in addition, that the degree of contraction depends directly upon the current density and that most of the contractile processes involved occur at the posterior part of the organism, since the anterior end continues to move forward, although at a reduced rate.

In order to bring out clearly the relation between the frequency with which these changes in rate occur in the same lot of amoebae, the percentage of specimens showing these phenomena was calculated from the individual records used in preparing Table IV. The

percentages are plotted against the corresponding current densities in Figure 6. By referring to this figure, it will be seen that in a current of 1δ, only about one-half of the specimens responded, i.e., 50 per cent showed anodal increase and 40 per cent cathodal decrease in rate; that, as the current density increased, the percentage of specimens responding increased; and that all the specimens showed anodal increase in a lower-current density than cathodal decrease. These results are similar to those obtained by Lund and Logan (1925) on coalescence of the cytoplasm of *Noctiluca* in various densities of current. The results presented in Figure 6 demonstrate, as has been so often done in the work of others, that to provoke a response, the current must exceed a certain minimal density or threshold; that the threshold varies for different individuals and for the two ends of the same individual; that it is less for anodal than for cathodal response; that it is attained for a greater percentage of specimens as the current density increases; and that it is ultimately attained for both ends of all specimens.

The foregoing results show clearly that, when subjected to a constant current, the ends of *Amoeba* undergo changes in form and in rate of movement; but they do not show what processes are involved in these phenomena. In order to ascertain more precisely the processes involved in these changes, numerous observations were made under high magnification with a Bausch and Lomb binocular compound microscope equipped with a 4 mm. objective and 15× compensating oculars. These observations were made as follows:

The amoebae used were washed as described above, and then mounted on a glass slide under a cover glass which was supported on ridges of vaseline. The cover glass was now pressed down until the drop of solution containing the amoebae filled the space beneath it; then the slide was put upon the stage of the microscope, after which the electrodes were brought near the ends of the cover glass. Contact was made between the electrodes and the solution with strips of moist filter paper. An active specimen was now selected, brought into focus, and movement carefully observed; then the current was made and the effect on the movement observed, after which the current was again broken and again made. This was repeated until the desired number of results was obtained.

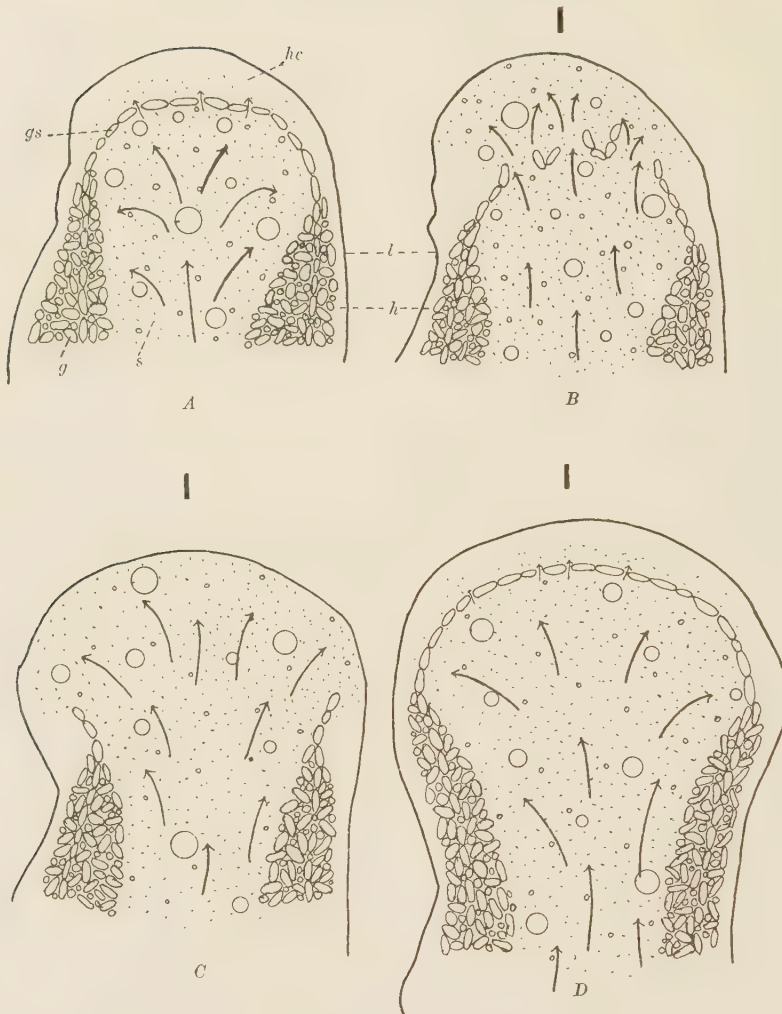


FIG. 7.—Sketches showing the changes produced by a galvanic current in the tip of a pseudopod of an amoeba which is advancing toward the cathode. *g*, Plasmagel; *s*, plasmasol; *l*, plasmalemma; *gs*, plasmagel sheet; *h*, hyaline layer; *hc*, hyaline cap; arrows, direction of flow of plasmasol; —, cathode. *A*, Anterior end showing the general relation of parts in normal locomotion (after Mast, 1926); *B*, showing break in the plasmagel sheet soon after the current is made with plasmasol streaming into the hyaline cap; *C* and *D*, later stages. Note that the plasmagel sheet disintegrates or breaks in more than one place. Note also that a new plasmagel sheet is not formed immediately, that the plasmagel tube is not extended as it is ordinarily, and that the plasmasol consequently spreads out laterally.

The results obtained are presented graphically in Figures 7 and 8. The sketches in Figure 7 represent different stages in the effect which the current produces at the anterior end of specimens moving toward the cathode. Before the current is turned on the pseudopod in monopodal specimens usually extends for considerable periods of time at a fairly uniform rate without any abrupt changes or spurts. During this time the plasmagel sheet gradually advances and remains unbroken (Fig. 7, *A*). Sometimes, however, it becomes stretched and suddenly breaks at some point, allowing the plasmasol to stream through into the hyaline cap. Then the plasmasol in contact with the plasmalemma gelates, forming a new plasmagel sheet, after which movement proceeds uniformly as before (Mast, 1926). When the current is made, the plasmagel sheet suddenly breaks, usually at several points. Since an amoeba is a turgid system, this local destruction of the plasmagel causes it to contract elsewhere, forcing the plasmasol into the hyaline cap (Fig. 7, *B*), where it spreads out laterally and, being fairly fluid, tends to assume a globular form, owing to the elasticity of the plasmalemma and the action of surface tension (Fig. 7, *C*). If the current is weak, gelation soon occurs, restoring the plasmagel sheet and the turgidity of the organism (Fig. 7, *D*). Now, however, instead of remaining intact for some time, as in unstimulated specimens, the plasmagel sheet breaks again almost immediately, after which it alternately forms and breaks at short intervals. If the current is strong or passes for some time, gelation is inhibited, preventing the formation of a new plasmagel sheet and the extension of the plasmagel tube forward. Meanwhile, the flow of plasmasol into the hyaline cap continues, with the result that, in a short time, the organism loses its monopodal form, becomes liquid and globular, and finally begins to disintegrate on the side facing the anode.

The sketches in Figure 8 represent different stages in the effect which the current produces at the posterior end of specimens moving toward the cathode. In unstimulated specimens, the plasmagel at the inner surface at the posterior end continuously solates and is forced forward by contraction of the plasmagel in that region (Fig. 8, *A*). Soon it arrives at the anterior end, where it again gelates either

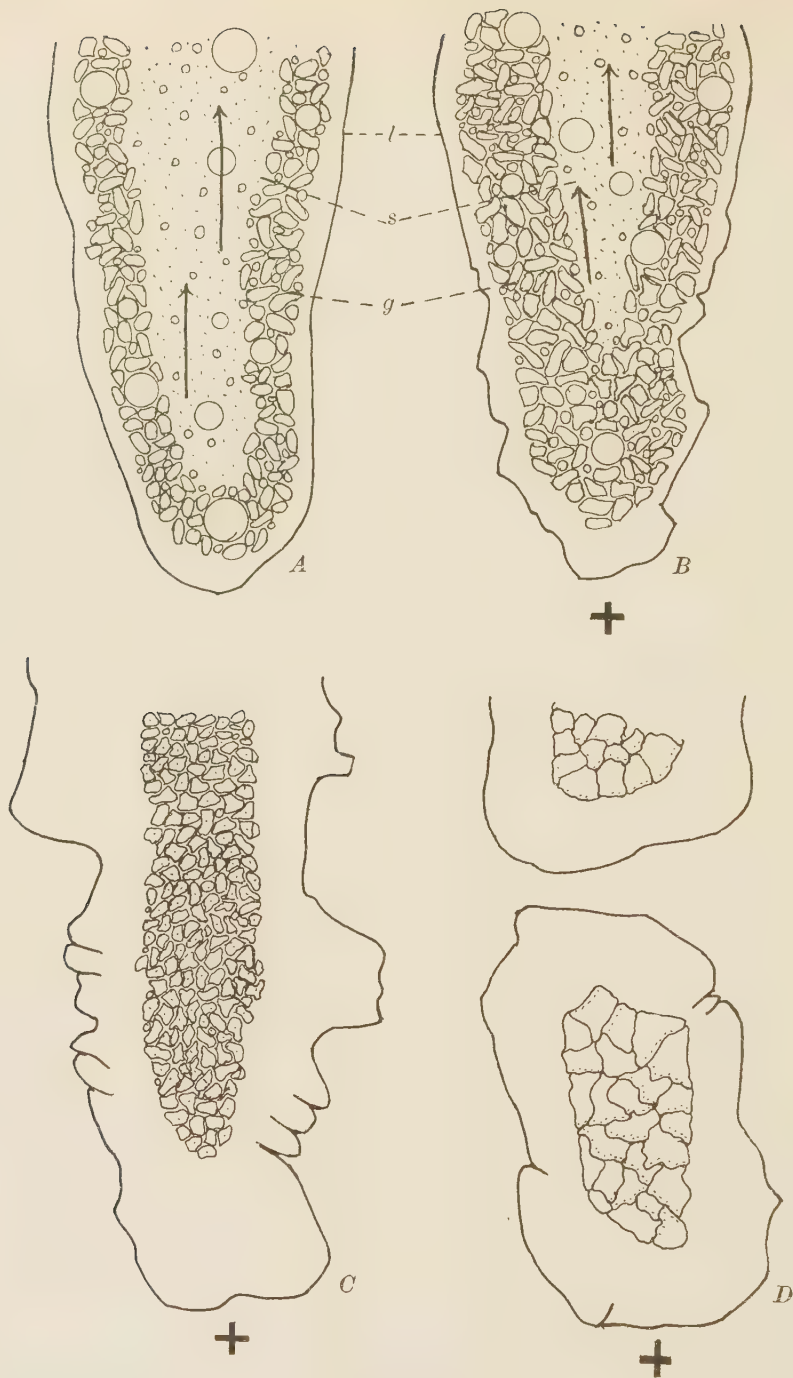


FIG. 8.—Sketches showing the changes produced by a galvanic current in the posterior end of an amoeba which is moving toward the cathode. Labels same as in Figure 7. *A*, Posterior end showing general relation of parts in normal locomotion; *B*, contraction of this end soon after the current is made, resulting in thickened plasmagel and in wrinkled plasmalemma; *C*, aggregation of the granules of the plasmagel into a dark compact mass, leaving the plasmalemma as a flaccid sac containing a hyaline liquid; *D*, pinched-off portion of the anodal end, showing the netlike structure of the plasmagel (*C* and *D* after Luce, 1927).

at the plasmagel sheet or at the forward edge of the plasmagel tube. When the current is made, the plasmagel at the posterior (anodal) end suddenly and rapidly contracts, an indirect effect following the rupture of the plasmagel sheet at the other end. If the current is weak, the period of rapid contraction continues while the plasmagel sheet is broken and ends when it is restored. If the current is strong or passes for some time, contraction of the plasmagel at the anodal end continues until the entire organism is drawn up into a dark mass, which ultimately disintegrates (Luce, 1927).

The results presented above indicate that the constant current alters the rate of locomotion in *Amoeba proteus* by definite polar actions at the ends of the organism; that these polar actions are strikingly different at the two ends; that decrease in rate of movement at the cathodal end is due to the destruction of the plasmagel sheet, allowing the pseudopod to expand laterally as well as to extend forward; that increase in rate at the anodal end is due to contraction or shrinkage of the plasmagel; that increase in the rate of locomotion of the organism as a whole is the result of increase in the frequency of breaks in the plasmagel sheet; and that the magnitude of these processes varies directly with the strength of the current.

THE REACTION-TIME IN THE RESPONSE TO ELECTRICITY

The experimental results presented in the preceding pages concern the effect of the constant current on the rate of locomotion in amoebae moving toward the cathode. In these experiments, however, owing to the fact that response to electricity occurs within a few seconds after the current is made, it was impossible to ascertain the reaction-time. It was accidentally discovered that this can be done in amoebae which are moving toward the anode.

The methods used in ascertaining the reaction-time were essentially like those used in the experiments described above. The amoebae were removed from a stock culture, transferred through four changes of test solution, and allowed to remain 15 minutes in the last for adjustment. Then two to five specimens were selected and transferred to test solution in the middle compartment on the observation slide. This compartment was now covered with a cover glass. As soon as the amoebae became attached, a specimen which was mono-

podal and moving toward the anode was selected; the circuit was then closed and the response observed.

In amoebae, which are monopodal and unstimulated, the plasmasol throughout the whole length of the organism flows toward the anterior end. If the current is made when they are moving toward the anode, the flow of the plasmasol changes; in a weak current it ceases and then reverses in direction at the cathodal end, with only a temporary cessation at the anodal end. The reversed flow continues only a few moments, after which it again reverses; streaming now proceeds in the original direction; later the organism gradually turns and becomes directed toward the cathode or rounds up and ceases to

TABLE V

REACTION-TIME IN A GIVEN SPECIMEN IN SUCCESSIVE RESPONSES TO A CURRENT OF 68 AT INTERVALS OF 1 MINUTE

Mean reaction-time, 1.145 seconds.

Standard deviation, 0.09 second, or 7.91 per cent.

Designation of Observation	Reaction-Time (Seconds)	Designation of Observation	Reaction-Time (Seconds)
1.....	1.1	6.....	1.2
2.....	1.0	7.....	1.1
3.....	1.2	8.....	1.2
4.....	1.15	9.....	1.2
5.....	1.0	10.....	1.3

move. In a strong current, the entire plasmasol stops flowing and then reverses in direction, resulting in complete reversal in direction of movement of the organism; i.e., it now moves toward the cathode. In either case, if the circuit is opened immediately after the reverse flow at the cathodal end begins, locomotion, when it is resumed, is in the original direction; i.e., toward the anode.

These changes in the flow of the plasmasol do not take place immediately after the circuit is closed; the period which intervenes between the application of the stimulus and the response is known as the "reaction-time." In the observations reported below, a monopodal amoeba moving toward the anode was stimulated with a current of known density and the reaction-time ascertained with a stop watch. Immediately after response, the circuit was opened; then 1 minute after the preceding stimulation it was closed again; that is,

the reaction-time plus the rest period equaled 1 minute. This was repeated until the desired number of results was obtained. The reaction-time at the two ends of *Amoeba* was investigated separately; that at the posterior (cathodal) end will be considered first.

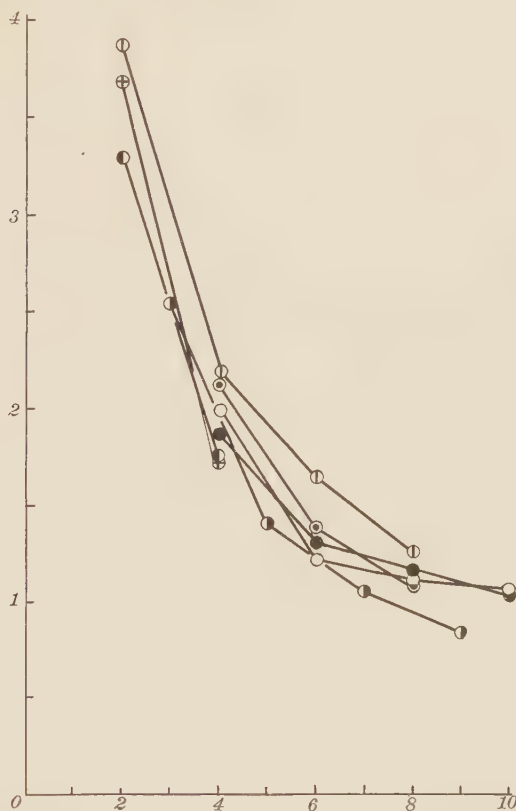


FIG. 9.—Graphs showing variation in the reaction-time in amoebae in currents of the same and of different densities. Abscissae, current density (δ); ordinates, reaction-time in seconds. Each curve is the record of one individual, and each point thereon is the average of three or more readings.

A. REACTION-TIME AT THE POSTERIOR (CATHODAL) END

1. *Individual variation in the reaction-time.*—The results obtained in a typical series of observations on the reaction-time at the posterior end of *Amoeba* are presented in Table V and Figure 9. By referring to this table, it will be seen that in ten consecutive trials the

mean reaction-time equaled 1.145 seconds, with a standard deviation of only 0.09 second, or 7.91 per cent. By referring to Figure 9, it will be seen that the reaction-time varied in different individuals in the same density; e.g., in a current of 6 δ , it ranged from 1.23 seconds to 1.65 seconds, with an equally great range at several other densities.

The results presented show that in currents of the same density there is considerable variation in the reaction-time in different individuals but surprisingly little in a given individual in successive trials.

2. *Relation between reaction-time and current density.*—Having now demonstrated that by measuring certain responses in amoebae which are moving toward the anode it is possible to obtain fairly consistent quantitative results concerning the reaction-time in the response to electricity, we are in a better position to ascertain precisely the relation between the reaction-time and the current density.

Before proceeding with this study, however, it seems necessary to digress long enough to describe the procedure followed in making the observations. Owing to the facts that individual variation in the reaction-time is marked and that only a small number of readings can be obtained on a single specimen before its direction of movement or its monopodal form changes, the following procedure was adopted: The reaction-time for an individual was first ascertained in each of three successive minutes in a current of known density in order to establish the average reaction-time for the individual in that density and then in each of the next three successive minutes in a stronger current in order to establish the change in reaction-time produced by the increase in the current. In this way the reaction-time for numerous specimens was ascertained in currents of various densities.

The results obtained are presented in Tables VI and VII and Figure 10, A. Table VI contains the results of all the tests in considerable detail. By referring to this table, it will be seen that the average reaction-time decreased as the current density increased; e.g., in 2 δ , it was 3.585 seconds; in 6 δ , 1.219 seconds; and in 10 δ , 0.858 second. It will also be seen that it was greater in low than in high temperatures; e.g., in 4 δ , it was 1.837 seconds at 19° and 1.758 sec-

onds at 25° ; and in 6 δ , it was 1.280 seconds at 19° and 1.225 seconds at 25° . It will be seen, moreover, that the reaction-time was greater in amoebae which had been previously stimulated by a current than

TABLE VI

RELATION BETWEEN CURRENT DENSITY AND REACTION-TIME AT
THE POSTERIOR END OF *Amoeba*

Each number represents the average for ten specimens; each pair of numbers joined by a vertical line, reaction-time on the same group of amoebae in currents of different densities; groups above slanting line, series of experiments performed at 18° – 21.5° C.; groups below slanting line, those performed at 22° – 27.5° C.

Current Density	Reaction-Time (Seconds)	Total Number of Readings	Average Reaction-Time (Seconds)
2	3.585	13	3.585
3	2.496	37	2.496
4	1.850 1.837	78	1.809
5	1.758 1.431	73	1.388
6	1.225 1.152 1.280	90	1.219
7	1.087 1.090	60	1.088
8	1.015 1.005	60	1.010
9	0.892	30	0.892
10	0.858	30	0.858

TABLE VII

RELATIONS BETWEEN CURRENT DENSITY AND REACTION-TIME AT THE
POSTERIOR (CATHODAL) END OF *Amoeba*

(1) Current Density (i) (Microamperes)	(2) Average Reaction-Time (t) (Seconds)	(3) Quantity of Current (it)	(4) Calculated Value of K Where $K = i\sqrt{t}$	(5) Calculated Value of K Where $K = i\sqrt{t} - (0.684i)$
2.....	3.585	7.170	3.786	2.418
3.....	2.496	7.488	4.737	2.685
4.....	1.809	7.236	5.380	2.644
5.....	1.388	6.940	5.890	2.470
6.....	1.219	7.314	6.624	2.520
7.....	1.088	7.616	7.301	2.513
8.....	1.010	8.080	8.040	2.568
9.....	0.892	8.028	8.496	2.340
10.....	0.858	8.580	9.260	2.420

in those which had not been so stimulated; e.g., in 4δ , it was 1.850 seconds with previous stimulation and 1.837 seconds without; and in 6δ , it was 1.225 seconds with, and 1.152 seconds without previous stimulation. It will furthermore be seen that these relations do not seem to hold in currents stronger than 6δ . It should, however, be mentioned that the response in *Amoeba* to currents weaker than 3δ is irregular and indefinite and that only a small percentage of the individuals respond at all, while in currents stronger than 6δ the reaction is too rapid to be measured accurately with a stop watch.

The results presented in this table seem to indicate that decrease in temperature and previous stimulation by electricity produce the same or similar changes in *Amoeba*, since both cause increase in reaction-time. This may be due to change in viscosity or to reduced excitability or to some other change. The effects of temperature and previous stimulation on the reaction-time merit more intensive investigation.

Table VII (cols. 1 and 2) contains the average of the results presented in detail in Table VI; they are plotted as a solid curve in Figure 10, *A*, with the ordinates as reaction-time and the abscissae as current density. By referring to the table and the figure, it will be seen that the reaction-time at the cathodal end of *Amoeba* decreases as the current increases and that the curve giving the relation between reaction-time and current density closely simulates the hyperbola of equation, $xy = 7$, presented in the figure by the broken curve.

The question now arises as to the quantity of electricity necessary to produce a response and the relation between this and the current density. The quantity of electricity necessary to produce a response is equal to the density of the current times the time the current must act to produce the response. The reaction-time measured above, however, is composed of two parts: the stimulation period, a time during which flow of current is necessary, and the latent period, a time during which flow of current is not necessary in order to produce a response. The reaction-time consequently does not represent the time necessary to produce a response; however, it probably closely approximates it. The product of this and the density (*it*) was consequently calculated. The results are given in Table VII, column 3.

By referring to this column, it will be seen that the product (*it*) was practically the same in all except the stronger currents tested and that the increase in these currents was probably due to the inac-

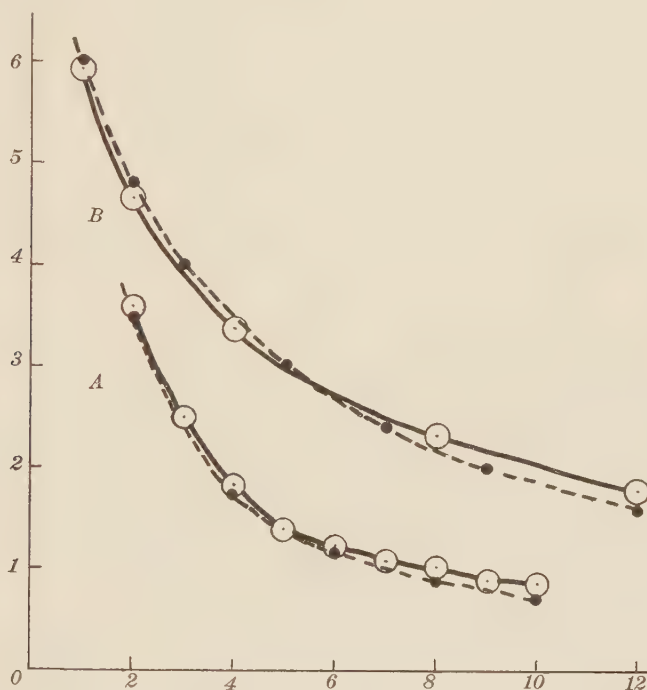


FIG. 10.—Curves showing the relation between reaction-time at the ends of amoebae and current density. Abscissae, current density (δ); ordinates, reaction-time in seconds. Solid curves, reaction-time; A, at posterior (cathodal) end, and B, at anterior (anodal) end; broken curves, hyperbolae; points on curves, points experimentally established. Note that the reaction-time decreases with increase in current density forming curves which closely simulate hyperbolae, and that the reaction-time for the anterior end is always longer than that for the posterior end in the same current.

curacy in the manipulation of the stop watch. We hope, in the near future, to ascertain the latent period over this range of densities, so as to obtain more accurately the relations in question.

It is of interest to see whether or not the response to the constant current in *Amoeba* is in accord with the law of electrical excitation which Nernst formulated in reference to response to electricity in striated muscle. His law states that for equal stimulating effect the

product of the intensity of the current (i) and the square root of its duration (t) is constant, i.e., ($i\sqrt{t}=K$). The value of K in this equation was calculated from the data presented above; it is given in Table VII, column 4. By referring to this column, it will be seen that the calculated value of K is not constant and that it increases as the current density increases. An attempt was then made to modify Nernst's equation so that the value of K would become constant. The results of this attempt are presented in the last column of the same table. This column shows that the value of K becomes nearly constant if the equation, $K=i\sqrt{t-(0.684i)}$, is used in the calculations. The number, 0.684, is the average interval between successive values of K (col. 4). The significance of this modifying factor is not known.

The tendency of the value of K to increase as the current density increases is evident also in the results of Kamada (1928) on the electro-destruction of the surface membrane of *Paramecium* and to a lesser extent in the results of Lund and Logan (1925) on coalescence of the protoplasmic films in *Noctiluca*. These cases may not, however, constitute real exceptions to Nernst's formula, since it is known that the formula only fits the results obtained in observations on muscles over a limited range of densities.

B. REACTION-TIME AT THE ANTERIOR (ANODAL) END

It was stated above that response at the anterior end in amoebae which are moving toward the anode consists in a temporary cessation of flow of plasmasol in weak currents and in cessation and then reversal in direction of flow in strong currents. This cessation does not take place immediately after the circuit is closed; i.e., there is a reaction-time.

The reaction-time at the anterior end was ascertained as in the preceding study. However, owing to the greater tendency of the anterior than of the posterior end to lose its monopodal form after stimulation, only one reading was obtained for each individual used. The reaction-time was ascertained for ten specimens in each density tested, and the average reaction-time in each calculated.

The results obtained are presented in Table VIII; they are plotted in Figure 10, *B*. By referring to the table and the figure, it will be

seen that the reaction-time decreases as the current density increases and that the curve giving the relation between reaction-time and current density closely simulates the hyperbola of equation, $xy + 3y = 24$, presented in the figure as a broken curve. It will also be seen that the value of K in Nernst's equation is not constant and that it increases as the current density increases. It will be seen, moreover, that if Nernst's equation is modified as described above, the value of K becomes more constant but not as constant as it does for the reaction-time at the posterior end. Greater irregularity in reaction-time would be expected at the anodal than at the cathodal end, since the response there is much less definite and precise.

TABLE VIII

RELATIONS BETWEEN CURRENT DENSITY AND REACTION-TIME AT THE ANTERIOR (ANODAL) END OF *Amoeba* AT 19.5°-22.0° C.

Current Density (i) (Microamperes)	Average Reaction- Time (t) (Seconds)	Quantity of Current (it)	Calculated Value of K Where $K = i\sqrt{t}$	Calculated Value of K Where $K = i\sqrt{t} - (0.824i)$
1.....	5.937	5.937	2.436	1.612
2.....	4.664	9.328	4.320	2.672
4.....	3.381	13.524	7.356	4.060
8.....	2.325	18.600	12.192	5.600
12.....	1.780	21.360	16.008	6.120
20.....	0.820	16.400	18.100	1.620

By comparing Table VII with Table VIII, or by comparing the two curves in Figure 10, it will be seen that the reaction-time at the posterior end was about 1 second less than that at the anterior end in each current density tested. This indicates clearly that the current produces its effect more quickly or more efficiently at the posterior (cathodal) than at the anterior (anodal) end of *Amoeba*, although the plasmagel is considerably thicker in the former than in the latter.

C. THE NATURE OF THE OBSERVED RESPONSE

The foregoing results support the view that the constant current causes solation of the plasmagel at the cathodal surface, but they do not show what occurs at the anodal surface. Moreover, they do not show the relation between the responses at the two ends of the organism. Accordingly, numerous observations were made to ascertain the nature and the relation of these responses.

The salient features in the results of these observations are presented in Figure 11, in which the sketches represent successive stages in the response to a constant current in *Amoeba*, as indicated by the

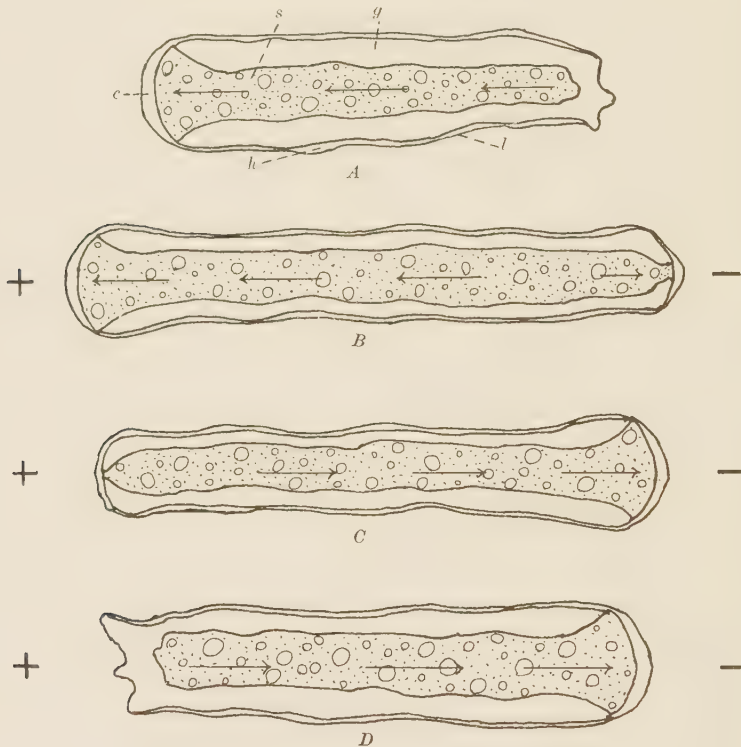


FIG. 11.—Sketches showing the changes produced by a galvanic current at the ends of an amoeba which is moving toward the anode. *A*, General relation of parts during normal locomotion; *B*, relation of parts soon after the current is made, showing reversal in the direction of flow of plasmasol at the cathodal end and continuation of the main flow toward the anode; *C*, relation of parts some time later, showing cessation of flow toward the anode and initiation of main flow toward the cathode; *D*, relation still later, showing the flow of the entire plasmasol toward the cathode. *g*, Plasmagel; *s*, plasmasol; *l*, plasmalemma; *c*, hyaline cap; *h*, hyaline layer; arrows, direction of flow of plasmasol; +, anode; —, cathode. Note that reversal in direction of flow may occur at the cathodal end before cessation of flow occurs at the anodal end.

direction of flow of the plasmasol. The following explanation is hypothetical, but it is in fair agreement with the observations.

In unstimulated specimens, the plasmasol throughout the whole

length of the organism flows toward the anterior end (Fig. 11, *A*) with solation occurring continuously at the posterior end and gelation occurring continuously at the anterior end. When the current is turned on, the thick layer of plasmagel at the posterior end, in specimens moving toward the anode, weakens and then breaks; now the plasmagel tube, being weak at both ends, contracts in the middle and forces plasmasol in both directions, thereby initiating the small reversed stream at the cathodal end and continuing the forward stream in other regions (Fig. 11, *B*). If the current is weak, gelation or fusion soon closes the plasmagel tube at the cathodal end, after which the whole column of plasmasol again flows in the original direction. Now the organism gradually turns around and proceeds toward the cathode. If the current is strong, gelation at the cathodal end is not sufficient to completely close the tube with a thick layer of gel. The flow of plasmasol toward the cathode persists, and the flow of plasmasol toward the anode soon ceases (Fig. 11, *C*). Then the end of the plasmagel tube toward the anode closes, owing to gelation or contraction, after which the whole column of plasmasol flows toward the cathode (Fig. 11, *D*).

The results presented show clearly that the constant current provokes response in *Amoeba* by definite polar actions at the ends of the organism; that destruction (solation) of the plasmagel is produced almost immediately at the cathodal end; that then contractive and later disintegrative processes are initiated at the anodal end; and that these responses are directly dependent upon the strength of the current.

DISCUSSION

The results presented demonstrate, as stated above, that the response to electricity in *Amoeba* is due to some polar action of the current which causes destruction (solation) of the plasmagel at the cathodal surface of the organism. It now remains to consider the question as to how this solation is produced.

The only immediate effects of passage of current which might produce solation of the plasmagel at the cathodal surface are (1) movement of ions and (2) movement of water.

Solation due to the movement of ions might be brought about

by the action of positive ions which are carried along by the current and accumulated within the plasmagel on the side toward the cathode. Under these conditions, we may assume that the accumulation of positive ions in some way increases the porosity of the plasmagel, since water passes or is forced through it and collects under the plasmalemma, forming a clear blister; that either the continued action of the cations or this passage of water so weakens the plasmagel that it finally breaks down completely, forming a pseudopod which extends toward the cathode; and that the accumulation of negative ions within the plasmagel on the side toward the anode decreases the porosity of the plasmagel in that region, resulting in increase in its elastic strength. Solation at the cathodal end and increase in elastic strength at the anodal end would result in the flow of plasmasol toward the cathode.

Solation due to the movement of water through the plasmagel as a capillary membrane might be brought about by the electroendosmotic action of the constant current. It is known that the more solid phase in protoplasm is negatively charged with respect to the more liquid phase and that the plasmagel behaves as a fairly stable membrane. If we consider that the plasmagel acts as a capillary membrane and that it is coated with an electrical double layer, then the charge on the plasmagel is negative and that on the surrounding film of water is positive. An external electromotive force applied to such a system will cause a relative motion of the two layers. Since the negative charge is imbedded in the plasmagel and does not move, the layer of liquid, being positively charged and freely movable, will pass through the plasmagel toward the negative pole. Indeed, the first visible response to a current in amoebae attached to the substratum consists of the passage of water through the plasmagel. The subsequent movement of small and then large granules indicates clearly that the plasmagel gradually increases in porosity until it finally breaks down completely.

The data presented in this paper do not reveal precisely which of these two possible actions of the constant current is responsible for solation of the plasmagel on the cathodal side. On the basis of the foregoing discussion we should expect that either the action of accumulated positive ions or of the electroendosmotic movement of

water would account for it, and that either of these processes would show a definite and consistent relation to the strength and duration of the current.

Mast (1931) found that solation occurs in specimens subjected to alternating current, in which electroendosmosis cannot be involved. It is evident therefore that movement of water plays only a minor rôle, if any, in solation produced by alternating current and that it must be largely, if not entirely, due to differential accumulation of ions. If this is true for alternating current, it probably is also true for direct current.

SUMMARY

1. The rate of locomotion in monopodal specimens of *Amoeba proteus* under constant conditions is fairly constant over long periods of time.

2. The nature of the response to a galvanic current in *Amoeba* depends upon the direction of movement when the current is made and upon the strength of the current.

3. Specimens moving toward the cathode respond to sudden increase in current density by increase in rate of movement at the posterior end and decrease in rate at the anterior end, resulting in contraction of the organism. These changes in rate, which vary in magnitude with the strength of the current, persist only a few moments and then gradually disappear.

4. Specimens moving toward the cathode respond to prolonged passage of a constant weak current by increase in the rate of locomotion, which persists for several minutes. They respond to prolonged passage of a strong current by contraction, which is followed by disintegration of the organism beginning on the anodal side.

5. Specimens moving toward the anode respond to sudden increase in current density by reversal in the direction of protoplasmic flow at the cathodal end, followed about a second later by cessation of flow at the anodal end.

- a) In weak currents, both are momentary, then movement is resumed in the original direction; in strong currents, both persist, and movement is continued in the opposite direction.

- b) The time between the closing of the circuit and the response, reaction-time, varies nearly inversely with the square of the current

density; the time-intensity relation in this response is, however, more nearly in accord with the equation, $i\sqrt{t} - (xi) = K$, than with Nernst's equation, $i\sqrt{t} = K$, in which i is the intensity of the current and t its duration.

c) Both decrease in temperature and previous stimulation by the current cause increase in the reaction-time. This may be due to increase in viscosity of the cytoplasm.

6. Response to the constant electric current in *Amoeba proteus* is due to local decrease in the elastic strength of the plasmagel on the cathodal side of the organism.

LITERATURE CITED

- BAYLISS, W. M. 1920. The properties of colloidal systems. IV. Reversible gelation in living protoplasm. Proc. Roy. Soc. London, **91**-B: 196-201.
- BECQUEREL, M. 1837. Influence de l'électricité sur la circulation du *Chara*. Compt. Rend. Acad. Sci. Paris, **5**: 784-88.
- BECQUEREL, M., and DUTROCHET. 1838. Influence de l'électricité sur la circulation du *Chara*. Ann. Sci. Nat., Ser. II, **9**: 80-87.
- BERSA, E., and WEBER, F. 1922. Reversible Viskositätserhöhung des Cytoplasmas unter der Einwirkung des elektrischen Stromes. Ber. deutsch. Bot. Ges., **40**: 254-58.
- CARLGREN, O. 1900. Über die Einwirkung des constanten galvanischen Stromes auf niedere Organismen. Arch. f. Anat. u. Physiol., Physiol. Abt., pp. 49-76.
- CHIFFLOT, J., and GAUTIER, C. 1905. Sur le mouvement intraprotoplasmique à forme brownienne des granulations cytoplasmiques. Bull. d. l. Soc. Nat. d. Saone-et-Loire. Also, Jour. de Bot., **19**: 40.
- CLARK, W. M. 1928. The determination of hydrogen ions. Baltimore: Williams & Wilkins. Pp. 717.
- COEHN, A., and BARRATT, W. 1905. Über Galvanotaxis vom Standpunkte der physikalischen Chemie. Zeitschr. f. allg. Physiol., **5**: 1-9.
- DAVENPORT, C. B. 1897. Experimental morphology. New York: MacMillan. Pp. 509.
- ENGELMANN, T. W. 1869. Beiträge zur Physiologie des Protoplasma. Arch. f. d. ges. Physiol., **2**: 307-22.
- EWART, A. J. 1903. On the physics and physiology of protoplasmic streaming in plants. London: Oxford. Pp. 131.
- HIRSCHFELD, L. 1909. Ein Versuch einige Lebenserscheinungen der Amöben physikalisch-chemisch zu erklären. Zeitschr. f. allg. Physiol., **9**: 529-34.

- HÖRMANN, G. 1898. Studien über die Protoplasmaströmung bei den Characeen. Jena. Pp. 79.
- HOPKINS, D. L. 1928. The effect of certain physical and chemical factors on locomotion and other life processes in *Amoeba proteus*. Jour. Morph. and Physiol., 45:97-119.
- JÜRGENSEN, T. 1861. Über die in den Zellen der *Vallisneria spiralis* stattfindenden Bewegungserscheinungen. Studien d. Physiol. Inst. Breslau, 1:87.
- KAMADA, T. 1928. The time-intensity factors in the electrodestruction of the membrane of *Paramecium*. Jour. Faculty of Science, Tokyo Imperial Univ., Sec. 4, 2:40-49.
- KOKETSU, R. 1923. Über die Wirkungen der elektrischen Reizung an den pflanzlichen Zellgebilden. Jour. Dept. Agri. Kyushu Imperial Univ., 1:1-133.
- KÜHNE, W. 1864. Untersuchungen über das Protoplasma und die Contractilität. Leipzig.
- LILLIE, R. S. 1923. Protoplasmic action and nervous action. Chicago: University of Chicago Press. Pp. 417.
- LUCE, R. H. 1926. Orientation to the electric current and to light in *Amoeba*. Anat. Rec., 32:55.
- . 1927. Response to light and electricity in *Amoeba*. Dissertation in Johns Hopkins Library. Pp. 134. Not yet published.
- LUND, E. J., and LOGAN, G. A. 1925. The relation of the stability of protoplasmic films in *Noctiluca* to the duration and intensity of an applied electric potential. Jour. Gen. Physiol., 7:461-71.
- MAST, S. O. 1926. Structure, movement, locomotion, and stimulation in *Amoeba*. Jour. Morph. and Physiol., 41:347-425.
- . 1927. Response to electricity in *Volvox* and the nature of galvanic stimulation. Zeitschr. f. vergl. Physiol., 5:739-61.
- . 1931. The nature of the action of electricity in producing response and injury in *Amoeba proteus* (Leidy) and the effect of electricity on the viscosity of protoplasm. *Ibid.*, 15:309-28.
- MCCLENDON, J. F. 1911. Ein Versuch, amöboide Bewegung als Folgeerscheinung des wechselnden elektrischen Polarisationszustandes der Plasmahaut zu erklären. Arch. f. d. ges. Physiol., 140:271-80.
- NERNST, W. 1908. Zur Theorie des elektrischen Reizes. *Ibid.*, 122:275-314.
- PANTIN, C. F. A. 1924. On the physiology of amoeboid movement. II. The effect of temperature. Brit. Jour. Exp. Biol., 1:519-38.
- SCHAEFFER, A. A. 1920. Amoeboid movement. Princeton: Princeton University Press. Pp. 156.
- SCHWITALLA, A. M. 1924. The influence of temperature on the rate of locomotion in *Amoeba*. I. Locomotion at diverse constant temperatures. Jour. Morph. and Physiol., 39:465-513.

- VELTEN, W. 1876. Einwirkung strömender Elektrizität auf die Bewegung des Protoplasma, auf den lebendigen und toten Zelleninhalt, sowie auf materielle Theilchen überhaupt. Sitzungsber. d. Akad. Wien, Mathem.-naturw. Kl., 73:343.
- VERWORN, M. 1896. Untersuchungen über die polare Erregung der lebendigen Substanz durch den constanten Strom. IV. Mittheilung. Arch. f. d. ges. Physiol., 65: 47-62.

VITA

William F. Hahnert was born near Logansport, Indiana, on October 6, 1901. He received his elementary education in a Washington Township district school and in the Logansport High School. He entered DePauw University in 1923, and received the degree of Bachelor of Arts magna cum laude in June, 1927.

In the fall of 1927 he entered the Johns Hopkins University as a student in Zoölogy. He was assistant in General Biology, 1927-28; and in General Physiology, 1928-30; and he held the Adam T. Bruce fellowship, 1930-31.

He spent the summer of 1926 as a student in the Marine Biological Laboratory, Woods Hole, Massachusetts. During the summer of 1929, he was research assistant to Professor S. O. Mast at the Mount Desert Island Biological Laboratory, Maine.

